RATE OF PHOSPHORUS UPTAKE BY PHAEODACTYLUM TRICORNUTUM [1]

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Research concerning phosphorus relations in unicellular algae has been active and profitable in several fields—oceanography, limnology, ecology, plant physiology, and biochemistry (Kamen and Spiegelman, 1948; Rice, 1953; Ketchum, 1954; Arnon, 1956; Bradley, 1957; Krauss, 1958; Steele, 1959; and many others). Two problems that have not yet been adequately studied are the uptake of phosphorus by given species of algae under controlled conditions, and the influence of algal populations on the recycling rates of phosphorus in whole communities. These problems are closely related, and ecologists recognize that the recycling rate is as fundamental a parameter of a community as the absolute abundance of algae and of nutrient phosphorus (Ketchum *et al.*, 1958). As an approach to the problem of measuring community recycling rates, we measured the rate at which *Phaeodactylum tricornutum* Bohlin (previously called *Nitzschia closterium* forma *minutissima* Allen and Nelson) (Lewin, 1958) accumulated both the abundant phosphate in fresh medium and the scant phosphate of P^{32} carrier.

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Methods

Chemical analysis. The particulate (cellular) fraction was separated from the dissolved phosphorus fractions by filtering samples of algal culture through either a $BaSO_4$ precipitate deposited on a sintered-glass filter funnel, or a millipore membrane filter, pore size 0.8 μ, previously washed by drawing 100 ml. of 10% HCl and several copious rinses of distilled water through it. Inorganic phosphate in samples of filtrate was determined by the Deniges-Atkins colorimetric method as described by Wooster and Rakestraw (1951). The color was measured in a photoelectric colorimeter (Ford, 1950) using a red filter (Corning 2408) and a 9- or 29-cm. light path. Total dissolved phosphorus in other samples of filtrate and cellular phosphorus caught upon the $BaSO_4$ precipitate or membrane filter were determined by the Harvey (1948) method as modified by Ketchum *et al.* (1955) using a 29-cm. path length in the photometer. Phosphorus concentration was calculated by use of appropriate factors obtained by calibration with standard solutions.

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Strickland and Parsons (1960) reported the limit of sensitivity of the phosphate method to be about 0.08 μg. at P/l (μg. at P/l = μM); the method for total phosphorus has about the same limit.

Radioisotope analysis. The dissolved P^{32} was separated from the cellular P^{32} fraction by filtering a 10-ml. portion through a membrane filter. The cells on the filter were not washed because such treatment leaches phosphorus from cells (Rice, 1953). To estimate the P^{32} held by sorption on the filter, we made "sorption" blanks by placing two or three membrane filter discs together on the filter holder so that the cells were caught on the upper disc and only the filtrate passed through the lower discs. The filter disc with the cells, the sorption discs, and 1-ml. aliquots of the filtrate were dried on separate planchets for counting with an end-window G-M detector connected to a scaler. Corrections were not necessary for self-absorption, for geometry of the detector, nor for coincidence losses, but corrections were made for decay when applicable.

The amount of vacuum applied to draw the water through the filter was one source of error in determining the P^{32} distribution between water and cells. To evaluate this, part of a culture of *Phaeodactylum* was freed of living cells by centrifuging it and then adding a drop of formalin to the centrifugate to kill any residual cells. To this, and to another portion of the culture containing living cells, equal amounts of P^{32} were added. Both were placed in the dark for more than an hour to permit equilibration with the P^{32}. When the centrifugate without living cells was drawn through three consecutive filters, only about 3% of the radioactivity was found on the filters, and most of that on the upper one (#1, Table I). Higher vacuum resulted in somewhat lower amounts of activity on the filters, presumably because less interstitial water remained. When the culture of living cells was filtered, practically all of the P^{32} was in the cells and was therefore retained by the top filter. The amount of activity in the filtrate increased with increased vacuum, and the activity on the sorption filters (#2 and 3) was quite variable (Table I). Although the amount of P^{32} on the sorption filters from the culture was much lower than that from the cell-free centrifugate, it was large compared to the P^{32} in the filtrate. The increasing P^{32} in the filtrate with intensified suction suggested physical damage to the cells, with labeled particles and soluble fractions being caught on the

TABLE I

Radioactivity detected on membrane filters and in the filtrate at different degrees of vacuum expressed as the differential between atmospheric and flask pressures. Values are counts per minute (cpm) in 10 ml. of centrifugate or culture

	Vacuum (mm. Hg):	50	100	200	250	360	510	660
Centrifugate:	Filter #1	460			450			330
	2	140			110			110
	3	140			120			90
	Filtrate	20,700			19,600			20,700
Culture:	Filter #1	18,200	18,200	18,400		18,000	18,200	18,600
	2	4.7	9.5	6.2		6.5	3.1	2.4
	3	4.4	5.3	2.8		1.4	2.0	1.9
	Filtrate	4.0	8.0	5.0		19	11	25

lower millipore filters and passing through with the filtrate. The measured activity in the filtrate of experiments I to V (Table II) is probably an overestimate because the importance of gentle vacuum was not then known; the suction used was usually in the range 250–500 mm. Hg. In experiments VI to X the pressure differential was kept below 50 mm. Hg. Because of the release of some phosphorus from the cells during filtration, the radioactivity on the sorption blank was added to the activity of the cells.

TABLE II

Comparison of concentration of phosphate in culture medium as measured chemically with that calculated from the minimum concentration of P^{32} during the experiment.

$$\text{Calculated } PO_4 = \frac{\text{minimum filtrate } P^{32}}{\text{total } P^{32}} \times \text{Total } P$$

Experiment	Chemical analysis (μM)		P^{32} (cpm/10 ml.)		Calculated filtrate PO_4 (μM)
	Filtrate* PO_4	Total P, cellular and dissolved	Filtrate minimum	Total, cellular and dissolved	
I	.11	1.77	10	4,180	.0042
II	.15	2.42	7	7,730	.0022
III	.16	4.06	4	7,890	.0021
IV	.16	4.79	8	7,720	.0050
V	.11	8.43	110	7,620	.12
VI	(.41)	1.37	32	47,500	.00093
VII	(.36)	1.62	23	52,000	.00072
VIII	(.50)	2.19	45	51,500	.0019
IX	(.50)	3.25	83	199,000	.0014
X	(.41)	5.63	80	203,000	.0022

* The results in parentheses are not considered dependable because of unusually large blanks.

Measurement of phosphate concentration by isotope partition. The partition of P^{32} between cells and medium was used to measure the dissolved phosphate concentration when it was near or below the limit of sensitivity of chemical analysis. Assuming that algae absorb $P^{32}O_4$ and $P^{31}O_4$ to the same degree, and that they do not release labeled phosphorus compounds other than orthophosphate, at equilibrium the ratio dissolved P^{32}/total P^{32} should equal the ratio dissolved PO_4/total P. Therefore, dissolved PO_4 = (total P) (dissolved P^{32})/(total P^{32}). This calculation undoubtedly overestimates the real concentration for the following reasons: (1) equilibrium, and hence the minimum dissolved P^{32}, may not have been reached when the experiment was terminated; (2) even gentle suction may damage some cells, releasing labeled phosphorus; and (3) the assumption of negligible release of labeled soluble organic phosphorus compounds during even short experiments may be false. The dissolved PO_4 calculated from P^{32} in nine cultures equilibrated in the dark was less than 4% of the value obtained by chemical analysis (Table II). Only in experiment V do the two values agree closely.

These results suggest that in media equilibrated with P-deficient algae the major part of the PO_4 measured chemically was liberated from acid-labile phosphorus compounds in the filtrate by the acid-molybdate reagent during the chemical analysis, and that only a small fraction was present as free phosphate ions in the whole

culture. The values in Table I show that phosphate from cells damaged during filtration would have been too low to detect chemically. We took the values derived from isotope partition to be the real phosphate concentrations and used them for further calculations.

Experimental Results

Uptake from phosphate-rich media. The net uptake of phosphorus by a pure culture of *Phaeodactylum* was studied by analytical chemical techniques. A culture was grown in constant light in "f-medium" (Guillard and Ryther, 1961) (Table III) until cell multiplication had reduced the phosphate concentration in the medium and the phosphorus content of the cells to a low level (4.2×10^{-15} mole/cell). Ten-ml. portions of this culture were added to each of four flasks containing 480 ml. of "f-medium" with various phosphate additions, giving 44×10^7 cells/liter in each flask. The dissolved phosphate concentrations were 8, 16, 32, and 80 μM (flasks A, B, C, and D, respectively). The flasks were illuminated (400 foot-candles) at 20° C., and 10-ml. samples were filtered periodically to measure dissolved and particulate phosphorus. The sensitivity and standard deviations were about 1 μM in samples containing low concentrations of phosphorus because of a 20-fold dilution during the analyses; at the highest concentrations the standard deviations were as much as 5 μM because dilutions as great as 100-fold were necessary. The cells were counted periodically using a Spencer Bright-Line counting chamber.

Table III

Composition of "f-medium" (Guillard and Ryther, 1961)

$NaNO_3$	150 mg. (1.76 mM)
$NaH_2PO_4 \cdot H_2O$	10 mg. (72.5 μM)
Fe sequestrene*	10 mg. (1.3 mg. Fe or 23.3 μM)
$Na_2SiO_3 \cdot 9H_2O$	30 mg. (3 mg. Si or 106 μM)
Vitamins:	
Thiamin·HCl	0.2 mg.
Biotin	1.0 μg
B_{12}	1.0 μg
Trace metals:	
$CuSO_4 \cdot 5H_2O$	0.0196 mg. (0.005 mg. Cu or 0.079 μM)
$ZnSO_4 \cdot 7H_2O$	0.044 mg. (0.01 mg. Zn or 0.153 μM)
$CoCl_2 \cdot 6H_2O$	0.020 mg. (0.005 mg. Co or 0.085 μM)
$MnCl_2 \cdot 4H_2O$	0.360 mg. (0.1 mg. Mn or 1.83 μM)
$Na_2MoO_4 \cdot 2H_2O$	0.013 mg. (0.005 mg. Mo or 0.052 μM)
Sea water	To one liter

* Sodium iron salt of ethylene dinitrilo tetraacetic acid (EDTA). Ferric chloride and EDTA or the sodium salt of EDTA can be mixed to give the same amounts of iron and the chelator; adjust pH to about 4.5. Ferric sequestrene is made by Geigy Industrial Chemicals, Saw Mill River Road, Ardsley, New York.

The time course curves of phosphate uptake by phosphorus-poor *Phaeodactylum* (Fig. 1A) are similar in shape, but the period of accumulation was much extended at high initial phosphate concentrations. Whereas it took 6 days before the phosphate was depleted in flask D, it was depleted in two hours or less in flask A. In

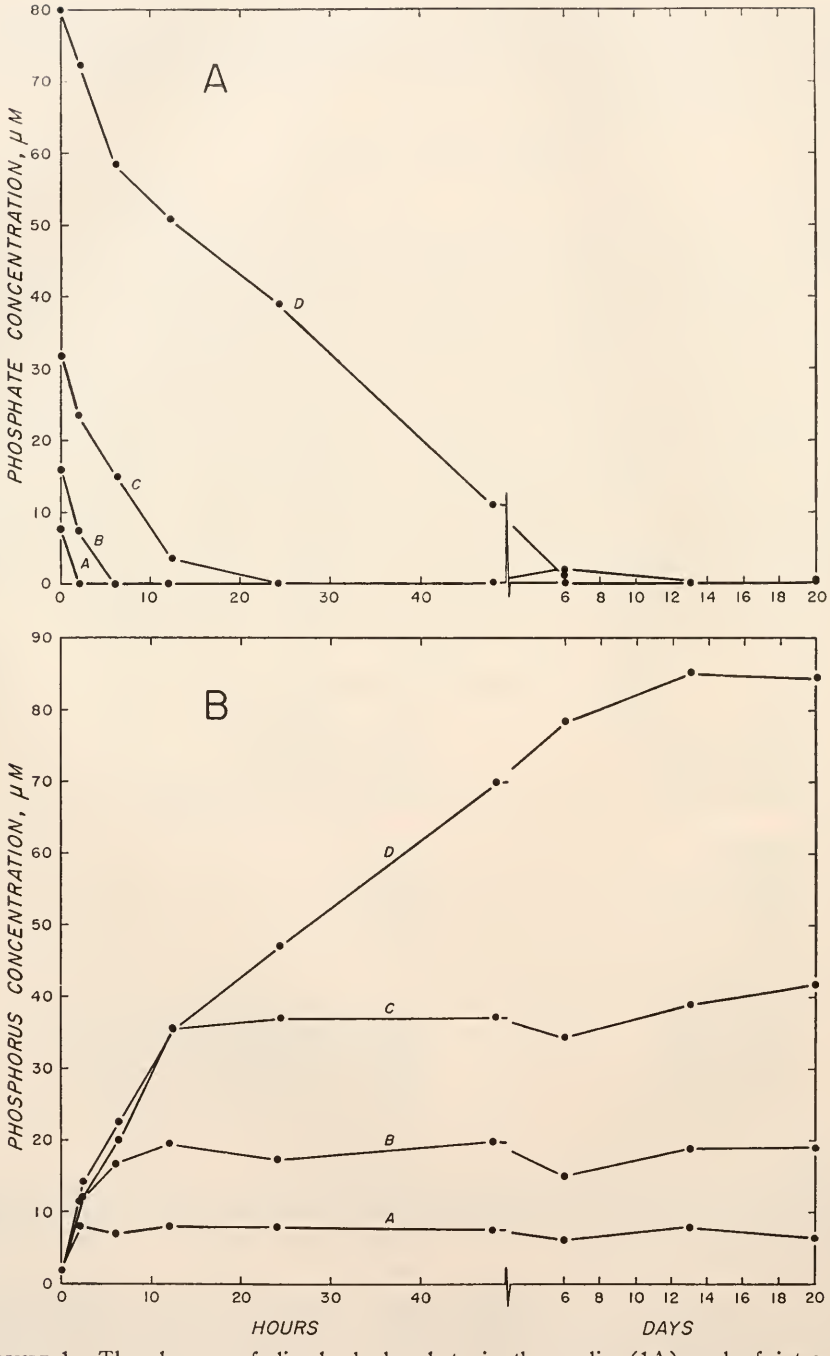

FIGURE 1. The changes of dissolved phosphate in the media (1A) and of intracellular phosphorus (1B) when *Phaeodactylum* was grown in media of different initial phosphate content. The initial cell count was 44×10^7 cells/liter and the initial phosphate concentrations were 8, 16, 32 and 80 μM, for A, B, C, D, respectively.

all cultures the phosphate was not significantly different from zero after 13 days. The phosphorus content of the cellular fraction (Fig. 1B) was the inverse of the phosphate content. The apparent increase in total phosphorus concentration at the end of the experiment is consistent with the usual rate of evaporation of water from media. The difference between total dissolved phosphorus and dissolved phosphate, frequently referred to as dissolved organic phosphorus, was never a significant amount in A or B, but reached a maximum of about 8 μM in flasks C and D at 6 and 12 hours, respectively. In all cultures it was not significantly different from zero after the sixth day.

The quantity of phosphorus per cell, shown in Figure 2, reached a maximum in two to 12 hours, the peak occurring later in the flasks with higher initial phosphate

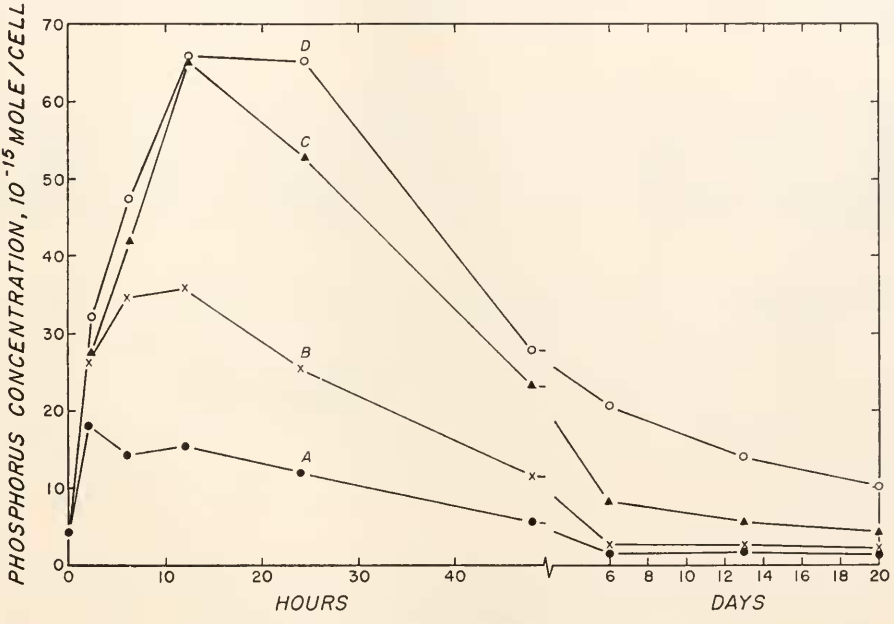

FIGURE 2. Phosphorus concentration of *Phaeodactylum* as a function of time after inoculation into media with varying initial phosphate concentrations as in Figure 1.

concentrations. The maximum concentration per cell in flasks A, B, and C was nearly a function of the initial phosphate concentration in the medium because these cultures were able to remove almost all of the phosphate before appreciable cell multiplication occurred. The number of cells in flask D, however, nearly doubled in the first 24 hours, at which time about half of the phosphate still remained in solution. These cells reached their peak concentration in 12 to 24 hours. The later decreases in phosphate per cell resulted from continuing cell multiplication.

The increase from 4 to 66 $\times$ 10^{-15} mole/cell (flasks C and D), a factor of 16, in 12 hours is remarkable. Part of the increase per cell may be attributed to cell enlargement prior to division, but much of the increase must represent a greater concentration of phosphorus in the protoplasm. Negligible uptake by adsorption was shown by an experiment described below. As growth of the culture continued, the

intracellular concentration declined until, at about 2×10^{-15} mole/cell in flask A, *Phaeodactylum* became phosphorus-deficient and stopped multiplying. In the other flasks cell division continued at a slow rate up to 20 days. The final cell counts varied from 400 to 960×10^7 cells/liter.

The rates of phosphate uptake by *Phaeodactylum* were neither linear, owing to removal of a constant quantity of phosphate per unit time, nor exponential, owing to removal of a constant proportion per unit time, but were intermediate. The linear, or mean, rates are simply the uptake of phosphate per cell divided by the duration of each sampling interval. The exponential rate at the beginning of each interval was calculated by

$$k = \frac{\ln P_t - \ln P_0}{n \cdot t} \tag{1}$$

and

$$v = k \cdot P_0 \tag{2}$$

where P_0 and P_t are the phosphate concentrations in solution at the beginning and end of a sampling interval, t is time in minutes, n is the cell density (number per volume), k is the velocity constant, and v is the instantaneous initial uptake rate. Both k and v are negative. The exponential and linear rates are shown in Table IV.

TABLE IV

Rates of phosphate uptake by Phaeodactylum in the light (400 fc) in media of high phosphate concentration. The first column for each experiment is the instantaneous initial rate calculated assuming an expontential rate of phosphate uptake; the second column is the mean rate assuming a linear uptake during the interval. Values are 10^{-17} mole/cell·minute

Initial PO_4 (μM): Approx. time (hours)	A		B		C		D	
	8		16		32		80	
	Initial	Mean	Initial	Mean	Initial	Mean	Initial	Mean
0–2	(32)*	13	24	17	15	13	13	12
2–6	—	—	(13)*	6	10	8	14	12
6–12	—	—	—	—	12	6	5	4
12–24	—	—	—	—	(1)*	1	3	3

* Undependable because of extremely low final phosphate concentration.

The agreement between flasks A to D during each time interval demonstrates that in all four experiments the rate of supply of PO_4 to the cells did not limit the uptake rates as long as PO_4 was chemically detectable. The rates were thus more dependent upon the physiological condition of the cells than upon the PO_4 concentrations. The decreasing rates in C and D as the cells became glutted (Fig. 2) after about 6 hours are also apparent.

The uptake of P^{32} by living, phosphorus-poor *Phaeodactylum* was compared to that of an equally dense suspension of chloroform-killed cells in darkness at 22° C. Separation by filtration showed that during the first hour the living cells accumulated 93% of the added P^{32} whereas the chloroform-killed cells took up only 3%.

The living cells continued their uptake, but the dead cells changed little during the next two hours. On the basis of this experiment and others in which cells were killed by heat, toluene, or chloroform, it was concluded that physical sorption by the cells was not an important factor in the rapid uptake of phosphorus by algal cultures.

Ketchum (1939a) showed *Phaeodactylum tricornutum* to be capable of accumulating 25×10^{-15} mole/cell in 48 hours of darkness. This value is an order of magnitude lower than the values in Table IV. The highest net uptake rates reported for *Phaeodactylum* by Ketchum (1939b), when the cells were added to medium containing about 1 μM phosphorus and illuminated, were about 20% of the rate of experiment A (Table IV) during the first two hours. His lower rates may be the result of the lower phosphate concentrations, the lower experimental temperature, the longer sampling interval, and perhaps a different physiological condition of the cells.

Uptake from phosphate-poor media. The rates of uptake at low phosphate concentrations were determined by adding P^{32} as phosphate to equilibrated cultures and measuring the rate of change of radioactivity in the medium. Sea water medium was prepared with five different concentrations of phosphorus and one-tenth the concentration of other nutrients of "f-medium." The phosphorus and iron were added aseptically after autoclaving to minimize formation of precipitates because such precipitates rapidly remove P^{32} from the water. Silicon was omitted from the medium since it does not limit growth of *Phaeodactylum*. Equal inocula from a phosphorus-poor culture were added to 500 ml. of each of the five media. The flasks were placed in darkness at 20° C. for 24 hours. Cell counts were made for each flask. Triplicate 100-ml. samples were then removed, and the cells were separated by filtration for phosphate, total dissolved phosphorus, and cellular phosphorus determinations. Practically all of the phosphorus at this time was in the cells (Table V). Radioactive phosphate was added to the remainder of each culture and they were kept in darkness at 21–25° C.

TABLE V

Phosphorus concentrations, cell counts, and uptake rates of Phaeodactylum in the dark in media of low phosphate concentrations

Experiment	Phosphorus concentration			Total P^{32} (10^5 cpm/l)	Cell count (10^7 cells/l)	Uptake rate (10^{-17} mole/cell min.)
	as PO_4 in filtrate (μM)	as cells in culture (μM)	within cells (10^{-15} mole/cell)			
I	0.054	1.8	6.1	4.4	29	3.0
II	0.052	2.4	10	8.2	23	2.7
III	0.052	4.1	18	8.5	23	2.2
IV	0.055	4.8	19	7.8	25	3.0
V	0.17	8.3	34	8.4	24	0.97
VI	0.011	1.2	8.2	48	15	1.3
VII	0.011	1.5	9.4	52	16	1.1
VIII	0.012	2.0	12	52	16	1.1
IX	0.041	3.1	19	200	16	3.2
X	0.042	5.5	34	210	16	3.0

Ten-ml. portions of the culture were filtered periodically during the next three hours and the P^{32} content of both the cells and the water was determined. The uptake of P^{32} by the cells proceeded smoothly and rapidly, the time course curves being logarithmic. It was from these data that we concluded that the chemical method of analysis overestimated the low concentrations of dissolved phosphate in the medium. The final equilibrium value in these cultures is given in the last column of Table II. This value plus the amount of carrier phosphate added with the P^{32} gives the original phosphate concentration listed in Table V. This assumes that the cells had reached equilibrium after the 24-hour dark period and reached the same equilibrium after assimilating the radio- and carrier phosphate during the three-hour experimental period. The amount of carrier phosphate added (0.01–0.05 μM) was large compared to the dissolved phosphate but small compared to the total phosphorus in the culture.

The rate of phosphate uptake was computed by a formula derived from (1) and (2):

$$v = \frac{\ln P_t^* - \ln P_0^*}{n \cdot t} \cdot P_0 \qquad (3)$$

in which P_0^* and P_t^* are the P^{32} concentrations in solution at the beginning and end of the interval and P_0 is the initial total dissolved phosphate content. The other symbols are as previously defined. The rates computed for the first 7–9 minutes of the experiment are given in Table V which also gives the total activity and the cell count for each culture.

The rates of uptake from the low phosphate concentrations by these cells are an order of magnitude less than those in Table IV where the cells were lower in phosphorus content and the dissolved phosphate concentrations were much greater. The rates at low concentrations showed no definite trend related to either the phosphate concentration or to the phosphorus content of the cell.

Discussion

The two experiments described were designed to measure phosphate uptake under contrasting conditions. In the first experiment (Table IV) phosphorus-deficient cells were transferred to a medium containing large amounts of phosphate relative to the amount in the cells. The different amounts formed a source of supply for periods ranging from about two hours to six days. The initial assimilation, when growth and cell division were negligible, increased the phosphorus content of the cells which were, thus, recovering from their deficiency. At longer times, which depended on the amount of phosphate made available, the content per cell decreased because of cell division. In this experiment the amount of phosphorus in the cell varied from 2 to 66×10^{-15} mole/cell, emphasizing again the wide range of variation which can be induced by varying the external conditions. The initial rates of assimilation under these conditions varied from 12 to 24×10^{-17} mole/cell·min. and decreased as the phosphate was removed from solution.

In the second experiment (Table V) phosphorus-deficient cells were allowed to assimilate varying amounts of added phosphorus in the dark so that at the start of the experimental period practically all of the phosphorus was in the cells, their content being from 6.1 to 34×10^{-15} mole/cell. Radio- and carrier phosphate was

added in amounts that increased the low concentrations of dissolved phosphate but that were small compared to the phosphorus in the cells. Under these conditions the uptake of phosphate varied from about 1 to 3×10^{-17} mole/cell·min. with no apparent relationship to either the cell phosphorus or the dissolved phosphate. Although the available phosphate concentration in the two experiments varied initially by nearly four orders of magnitude, the rates of assimilation differed by only one.

Phosphorus is not only assimilated rapidly by deficient cells, but intracellular phosphorus is also constantly exchanged with that in the water (Kamen and Spiegelman, 1948; Goldberg *et al.*, 1951; Rice, 1953; and Knauss and Porter, 1954). It was not possible to separate the two mechanisms, uptake and exchange, in the present experiments. The results given in Table V for the assimilation from low phosphate medium were calculated assuming all of the change was uptake with no exchange. A calculation was also made assuming that all can be attributed to exchange, with no net uptake (Russell, 1958), and this gave almost exactly the same rates.

Phaeodactylum stops dividing when the intracellular phosphorus falls to about 2×10^{-15} mole/cell. During its most active growth a population can double in about 18 hours of continuous illumination. The lowest rate of uptake we have measured, 10^{-17} mole/cell·min., would permit a phosphorus-deficient cell to double its phosphorus content in 200 minutes, or slightly over three hours. Since this rate of uptake was from very low phosphate concentrations in the medium, it seems unlikely that the rate of assimilation of phosphorus would ever limit the rate of growth of a phytoplankton population in nature. The total supply could, of course, determine the final size of the population.

The rate of uptake under the different conditions must reflect the physiological state of the cells and must ultimately be dependent upon both the supply of phosphorus in the cells and in the medium, although these relationships are not conclusively demonstrated in our experiments. On the other hand, if availability of intracellular energy and enzyme reserves, needed for the work of active transport through the cell membrane, strongly influences the uptake rate, then darkened cells can have rates nearly those of illuminated ones if they can draw upon previously stored reserves. Odum *et al.* (1958) similarly showed that illumination had little effect upon the P^{32} uptake of benthic algae.

Munk and Riley (1952) showed theoretically that small cells should absorb nutrients more rapidly than larger ones, and Odum *et al.* (1958) demonstrated that filamentous or thin benthic algae absorb P^{32} much faster than the more massive algae. There were, undoubtedly, some differences in cell size and surface-to-volume ratio in *Phaeodactylum,* but these differences probably affected the uptake rates only slightly.

In *Phaeodactylum* the chromatophore shrinks to one-third or less of its original length as the cell becomes phosphorus-deficient. Chlorophyll measurements in other experiments (unpublished) prove that the absolute amount of chlorophyll per cell declines markedly in phosphorus-deficient cells (*cf.* Ketchum *et al.*, 1958). Uptake rates per unit of protoplasm, cell nitrogen, or chlorophyll may prove to be more meaningful than per cell, especially when comparing the rates of algae of widely differing sizes or when studying natural mixed populations. In experiments VI–X, chlorophyll concentration was measured; it was initially 7.1 ± 0.6

$\times 10^{-14}$ g. Chl A/cell, and the uptake rates on this basis range from 1.6×10^{-4} to 4.5×10^{-4} mole P/g. Chl A·min.

Phaeodactylum is able to remove phosphate to levels below the sensitivity of the chemical analytical method. The lowest concentration, determined from P^{32} remaining in solution, was 7.2×10^{-10} *M*. This confirms the often quoted ability of algal cells to concentrate phosphorus greatly. We have not measured the volume of *Phaeodactylum* cells, but Ketchum and Redfield (1949) give a dry weight per cell of 2.32×10^{-11} grams. Assuming 20% dry matter in the cells and a density of 1, the volume of cells in experiments VI–X (Table V) would be 1.9×10^{-2} ml./l. Lewin *et al.* (1958) found very similar weights and volumes in fusiform *Phaeodactylum*. The concentration of phosphorus within the cells varied from 0.08 to 0.3 *M*. These were in equilibrium with external concentrations of 7.2 to 22×10^{-10} *M* at the end of the experiment (Table II), producing concentration factors of about 10^8. Under these conditions most of the intracellular phosphorus probably is firmly bound in the cells with only a very small fraction present as free phosphate ions.

The physiological condition of natural phytoplankton with regard to nutrients is still difficult to assess. The uptake rate even at low concentrations is high enough so that phosphorus should never be limiting in any waters with chemically detectable phosphate concentrations. Our experiments have shown, however, that the chemical method in use measures some materials which are not treated as free phosphate ions by the living cell. Also, *Phaeodactylum* can accumulate thirty times as much phosphorus as the minimum required for cell division and may reduce the concentration of phosphate to undetectable levels in the water while the cells are still relatively phosphorus-rich. Such cells can continue to divide, in the light, with no further phosphorus accumulation. Inability to measure phosphate in sea water, then, can not be taken as evidence that it is limiting population growth or organic production.

Summary

Portions of a phosphorus-deficient culture of *Phaeodactylum tricornutum* Bohlin were dispensed into fresh media containing phosphate concentrations from 8 to 80 μM. The instantaneous initial phosphate uptake rates were 12 to 24×10^{-17} mole/cell·min. The concentrations of phosphorus in the cells extended from a high of 66×10^{-15} mole/cell after 12 hours' exposure to phosphate-rich (32 and 80 μM) media to a low of 2×10^{-15} mole/cell when PO_4 depletion of the medium limited further growth. In another experiment *Phaeodactylum* was prepared with varying intracellular P concentrations in media with very low PO_4 concentrations. Radioactive phosphate was then added, the time course of P^{32} distribution was followed, and the rate of phosphate uptake was calculated. The initial rates ranged from 10^{-17} to 3×10^{-17} mole/cell·min. These were about one order of magnitude less than the uptake rates during the first two hours of the first experiment, even though the PO_4 concentrations were two to four orders of magnitude lower. Radioisotope analysis showed that *Phaeodactylum* decreased the phosphate in the medium to as little as 7.2×10^{-10} *M*, a concentration much below the limit of sensitivity of the chemical analytical method.

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